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THE EFFECTS OF PROTEASE INHIBITORS ON HISTIDINE DECARBOXYLASE ACTIVITIES AND ASSAY OF ENZYME IN VARIOUS RAT TISSUES

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Summary

Histidine decarboxylase (L-histidine carboxy-lyase, EC 4.1.1.22) of an extract of rat stomach was inactivated by a pancreatic extract. This inactivation was prevented by the protease inhibitors leupeptin, antipain, chymostatin, pepstatin, Trasylol and phenylmethanesulfonyl fluoride. Leupeptin, antipain, chymostatin and pepstatin together and phenylmethanesulfonyl fluoride alone prevented complete inactivation of the enzyme, while Trasylol had a weak protective effect. The inactivation and protection of histidine decarboxylase purified from whole fetal rats were similar to those of the stomach enzyme: both enzymes were strongly inactivated by trypsin and chymotrypsin, but not by elastase or carboxypeptidase Y. The histidine decarboxylase activities of various rat tissues were assayed in the presence of protease inhibitors: activity was highest in mast cells followed by the whole bodies of fetal rats and the stomach, while the activities were lower in decreasing order in the brain, spleen, lung and liver. Heart and kidney had no activity.

Introduction

Histidine decarboxylase (L-histidine carboxy-lyase, EC 4.1.1.22) is involved in the biogenesis of histamine from its precursor amino acid, L-histidine. The activities of this enzyme are very low in most tissues and the enzyme is so unstable that it has not been purified completely or characterized extensively [1—8]. Recently, we purified histidine decarboxylase from whole fetal rats and raised an antibody against it [9,10]. The precipitin lines between the antibody

and the enzymes from whole fetal rats and rat brain fused with spur formation [10]. In an attempt to purify histidine decarboxylases from various tissues for immunochemical comparison, we found that the enzyme in rat stomach was strongly inactivated under certain conditions. Subsequently, we found that the enzyme in whole bodies of mice was very difficult to assay unless protease inhibitors were included during the preparation of crude extracts and assay of activity [11]. This suggested that inactivation of the stomach enzyme might be due to contamination with pancreatic tissue, because while collecting large amounts of rat stomach for enzyme purification we did not separate the stomach carefully from surrounding tissues. Therefore, we investigated the effects of protease inhibitors on the histidine decarboxylase activity of rat stomach in detail. This paper reports the inactivation of the enzyme by pancreatic proteases and the effects of protease inhibitors in preventing this inactivation. The protective effects of protease inhibitors made it possible to assay very low activities of the enzyme in various tissues of rat.

Materials and Methods

Preparations of crude extracts. For inactivation studies, crude extracts of the stomach and pancreas of Sprague-Dawley rats were prepared as described previously in the absence of protease inhibitors [11]. Crude extracts of various tissues were prepared similarly for assay of histidine decarboxylase, except that protease inhibitors were added. Mast cells were collected from the peritoneal cavity of rats by the method of Ritchie et al. [12] and the enzyme was extracted as described above after treatment with 0.1% Triton X-100 at 4°C for 30 min.

Partial purification of histidine decarboxylase from whole fetal rats. Histidine decarboxylase was purified from the whole bodies of fetal rats as reported previously and the preparation at the Bio-Gel A-0.5 m step was used [9].

Assay of histidine decarboxylase. Histidine decarboxylase was assayed by the *o*-phthalaldehyde method [13] as reported previously with or without protease inhibitors [11].

Miscellaneous. Aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1) was assayed by the method of Karmen [14] with some modifications [15], and 3,4-dihydroxyphenylalanine decarboxylase (aromatic-L-amino-acid carboxy-lyase EC 4.1.1.28) as described previously [9]. Trasylol was obtained from Bayer, Leverkusen. Phenylmethanesulfonyl fluoride was from Sigma Chemical Co., Saint Louis, and was dissolved in 5% isopropyl alcohol. The other chemicals and methods used were as described previously [11].

Results and Discussion

Inactivaton of rat stomach histidine decarboxylase by pancreatic extract

During the purification of histidine decarboxylase from a large number of rat stomachs (Yamada, M. et al., unpublished data) by the procedure used for purification of enzyme from whole fetal rats [9], the enzyme was found to be completely inactivated after $(NH_4)_2SO_4$ fractionation followed by dialysis.

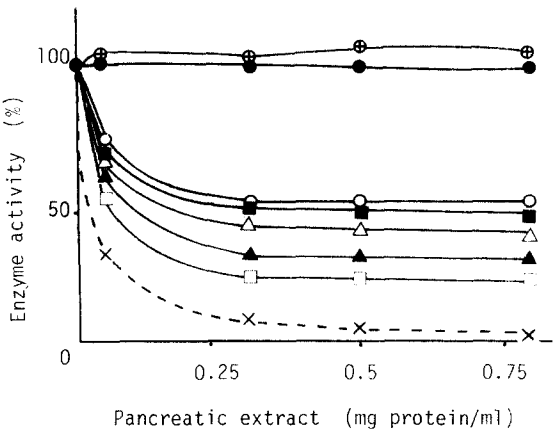

Fig. 3. Protection by protease inhibitors against inactivation of histidine decarboxylase by pancreatic extract. Rat pancreatic extract (0—800 μg protein) was incubated with 20 μg/ml pepstatin (□———□); antipain (▲———▲); leupeptin (■———■); chymostatin (△———△); a combination of these four inhibitors (⊕———⊕); 400 μg/ml Trasylol (○———○); 100 μg/ml phenylmethanesulfonyl fluoride (●———●) or no inhibitor (X- - - - -X), for 30 min in 0.8 ml Solution A at 37°C and then the mixtures were incubated with 0.1 ml rat stomach enzyme (1.5 mg, 300 pmol/min activity) for 2 h. Then 0.1 ml 2.5 mM L-histidine was added and the reaction was carried out for 60 min. Enzyme activity was measured as described for Fig. 1. Solution A is defined in the legend to Fig. 1.

enzyme was inactivated in a similar manner as the stomach enzyme (data not shown). Histidine decarboxylase seems to be more sensitive than other enzymes to proteases because no striking inactivation of aspartate aminotransferase of rat liver, or 3,4-dihydroxyphenylalanine decarboxylase of the whole bodies of fetal rats was observed under these conditions.

Histidine decarboxylase activities of various rat tissues

On the basis of the results described above, the histidine decarboxylase activities of various rat tissues were assayed in the presence of protease inhibitors. Although phenylmethanesulfonyl fluoride was as effective as a combination of

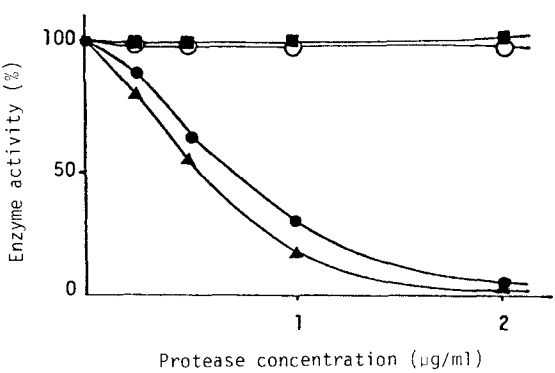

Fig. 4. Inactivation of rat stomach histidine decarboxylase by various proteases. The enzyme of rat stomach (1 mg, 250 pmol/min activity) was preincubated with various amounts of trypsin (●———●); chymotrypsin (▲———▲); elastase (■———■) and carboxypeptidase Y (○———○) for 2 h and then enzyme activity was assayed as described for Fig. 1.

TABLE I

HISTIDINE DECARBOXYLASE ACTIVITIES IN VARIOUS RAT TISSUES

Tissue	Enzyme activity (pmol/min per mg protein ± S.D.)
Brain	0.76 ± 0.51 (n = 5)
Heart	0 (n = 8)
Lung	0.07 ± 0.03 (n = 13)
Liver	0.01 ± 0.006 (n = 10)
Stomach	10.4 ± 4.3 (n = 10)
Spleen	0.13 ± 0.03 (n = 15)
Kidney	0 (n = 8)
Mast cells	118 ± 36 (n = 10)
Whole foetus	16.6 ± 7.7 (n = 16)

chymostatin, leupeptin, antipain and pepstatin in protecting the stomach enzyme against the pancreatic extract, a combination of the four inhibitors was used in measurement of the enzyme activity in crude extracts of various rat tissues, because these tissues may contain many kinds of proteases that are insensitive to phenylmethanesulfonyl fluoride, such as pepsin and cathepsin D. Pepstatin is a very potent inhibitor of acid proteases [19]. In assay of the activity in crude extracts of the spleen and lung, the presence of protease inhibitors was essential. As with extracts of the whole bodies of mice [11], extracts of the spleen and lung gave high blank values in the absence of a substrate. As shown in Table I, the highest activity was found in mast cells, moderate activity in the whole bodies of fetal rats and in the stomach, and lower activities in decreasing order in the brain, spleen, lung and liver. No activity was detected in the heart or kidney.

Activity in the mast cell extract was the highest ever reported, being 118 pmol/min per mg. A value of 20 pmol/min per mg was calculated from the results in Fig. 7 of the paper of Ritchie and Levy [12], assuming that their yield of mast cells was similar to ours. Under our conditions, the reaction of the enzyme in the mast cell extract proceeded linearly only with less than 20 μg protein and an incubation period of less than 20 min. When histamine was added to the reaction medium, nearly 100% was recovered, indicating that no further degradation of histamine occurred during the incubation. It is unknown why the reaction did not proceed linearly with more enzyme and a longer incubation period; possibly histidine decarboxylase in mast cells is extremely labile even in the presence of protease inhibitors, or possibly it is inhibited by some other factor extracted from the mast cells.

Histidine decarboxylase is the sole enzyme involved in the formation of histamine from its precursor amino acid, L-histidine. The regulatory processes of this enzyme are not known, and the ease with which the enzyme is inactivated by proteases might be one regulatory process. In this sense, it is interesting to test the effect of mast cell serine protease reported by Katunuma and co-workers [20], on the histidine decarboxylase of mast cells.

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